Effects of two prototypic polychlorinated biphenyls (PCBs) on lipid composition of rat liver and serum

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Mature male Sprague-Dawley rats received a single IP injection of either 2,2',4,4',5,5'-hexachlorobiphenyl (HCB), 3,3',4,4'-tetrachlorobiphenyl (TCB) (300 μm/kg) in corn oil (10 ml/kg) or the corn oil vehicle alone, and were killed four days later after having been fasted overnight. The vehicle control group consisted of rats which were allowed free access to feed as well as pair-fed animals. Lipid analyses were conducted on liver, hepatic microsomes and serum. TCB- (but no HCB-) treatment resulted in a statistically significant increase in total liver lipids and triglycerides. Liver phospholipids remained unchanged. Both PCBs increased the cholestrol and phospholipids content of the liver microsomal fraction. Serum lipids measured were not statistically different from control values. While HCB had little effect on the fatty acid composition of liver lipids, TCB caused an increase in C 18:1 (n-9) and a decrease in C 20:4 (n-6). Both PCBs increased C 18:0 in the hepatic microsomal fraction, but TCB also decreased C 16:0. Neither PCB altered the fatty acid composition of serum total lipids. These data are consistent with the concept that specific alterations in lipid metabolism are dependent on the structure of the PCB.

Keywords: liver; serum; lipid; polychlorinated biphenyls

Introduction

Because of their chemical inertness and physical properties, polychlorinated biphenyls (PCBs) have been widely used for industrial purposes (dielectric fluids, flame retardants, plastic additives, etc.), until they were detected as major environmental pollutants. Their toxicity is manifested as alterations of fundamental metabolic functions (respiratory, endocrine, reproductive) and in dermal lesions.[1] Particular effects of these compounds are hepatic damage (porphyria, liver enlargement, lipid infiltration) and changes in lipid metabolism, observed in several animal species (chicken, rabbits, rats, mice). Over the past fifteen years, a number of studies have reported an increase in liver and hepatic microsomal lipids (total lipids, phospholipids, neutral lipids and cholesterol) following PCBs administration.[2-5] This was confirmed by morphological observations of higher occurrence of lipid material, both in endoplasmic reticulum and in cytoplasmic droplets.[3] Serum lipids were also shown to be affected by PCBs which apparently modify the regulatory mechanisms of synthesis and degradation of cholesterol.[6,7]

The studies mentioned above were carried out with commercial PCBs, which are mixtures of many chlorinated biphenyls. The properties of the PCBs, especially on the induction of the hepatic monooxygenase system, are dependent on the structure of the molecule.[8] Briefly, the most acutely toxic PCBs are the ones with at least two adjacent halogens in the lateral positions of the biphenyl molecule and an absence of halogens in the ortho positions, a substitution pattern which favors a more coplanar conformation.[9]

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These PCBs resemble 3-methylcholanthrene in their ability to induce the cytochrome P-450, activity of aryl hydrocarbon hydroxylase and they more avidly bind a cytosolic receptor protein. In contrast, the ortho-substituted PCBs produce a phenobarbital-type of induction of cytochrome P-450 and associated aminopyrine-N-demethylase activity. As reported by a large number of studies, the structure and type of induction of the different PCBs seem to be related to their toxicity and mechanism of action.[10,11]

Data related to the effects of PCBs on lipid metabolism have found that the degree of chlorination of the mixture was related to the lipid accumulation in rat liver.[12] Aroclor 1016 and Aroclor 1242, two industrial mixtures with different chlorine contents, led to different effects on phospholipids and triglycerides in Chinese hamster ovary cells in culture.[13] But the degree of chlorination of the PCB molecule is not sufficient to explain the different effects of various compounds. Some studies investigating the role of pure PCB congeners on lipid metabolism indicated that the structure of the molecule is more related to the observed effects. In vitro studies where the PCB molecule was incorporated into liposomes showed that the perturbation which increases membrane fluidity was higher with ortho-substituted PCBs as compared with 3,3',4,4'-tetrachlorobiphenyl, a coplanar compound, or unsubstituted biphenyl ring.[14] Kohli and coworkers observed that a noncoplanar hexachlorobiphenyl, 2,2',3,3',5,5'-hexachlorobiphenyl, increased the phospholipid content of rat liver and did not alter triglyceride content, whereas the coplanar isomer, 3,3',4,4',5,5'-hexachlorobiphenyl, showed the opposite effects.[15] Moreover, the localization of lipid accumulation induced by the compounds was different.[16]

The present study was designed to investigate the effects of two pure PCB compounds on rat liver lipid composition. We used 3,3',4,4'-tetrachlorobiphenyl (TCB), a 3-methylcholanthrene-type of inducer of cytochrome P-450 and 2,2',4,4',5,5'-hexachlorobiphenyl, a phenobarbital-type inducer, to compare their effects on rat liver lipid composition.

Materials and methods

Chemicals

TCB (3,3',4,4'-tetrachlorobiphenyl) and HCB (2,2',4,4',5,5'-hexachlorobiphenyls) were synthesized from the corresponding halogenated benzidines, 3,3'-dichloro- and 2,2',5,5'-tetrachlorobenzidines, as previously described.[17]

Animals

Male Sprague-Dawley rats (Charles Rivers, France) were acclimatized for 3 weeks in a temperature-controlled room (20 ± 2°C), with a 12 hours light/dark cycle and fed a semi-synthetic diet.* At the age of 10 weeks, they were divided in several homogeneous groups of 8 animals and received an i.p. injection of either TCB, HCB (300 μmol/kg) or vehicle (corn oil: 10 ml/kg). Because TCB is known to reduce the food intake,[1] a control group was pair-fed and received the mean amount of food eaten by the TCB-treated animals the previous day. Another control group of 8 rats was fed ad libitum. This procedure allowed the comparison between TCB-treated rats and pair-fed control rats, and between HCB-treated rats and ad libitum control rats. Animals were killed by decapitation 4 days after xenobiotic administration. They were fasted overnight before killing. Liver was carefully excised and weighed; one portion was lyophilized and a second one homogenized in 0.25 M sucrose, buffered with 0.05 M Tric-HCl containing 0.1 mM EDTA (pH 7.4). The homogenate was centrifuged 20 min at 9000*g* at 4°C, then the supernatant was centrifuged again for 60 min at 105,000*g* at 4°C to obtain a microsomal pellet which was stored at −80°C until analysis. Blood was allowed to clot before centrifugation, serum was collected and was stored at −80°C.

Lipid analysis

Lipids of the lyophilized liver and of microsomes were extracted according to the method of Folch.[18] Neutral lipids and phospholipids were separated on silica cartridges and eluted successively with 20 ml of chloroform (triglycerides) and 20 ml of methanol (phospholipids).[19] After transmethylation with 14% boron fluoride,[20] methyl esters of fatty acids were analyzed by gas liquid chromatography (GLC) (Packard 427) on a glass capillary column (D: 0.3 mm; L: 45m), impregnated with 0.5% FFAP using a flame ionization detector). After extraction of liver microsomal lipids, cholesterol was assayed by CHOD–PAP (Boehringer), and phospholipids according to Bartlett[21]; triglycerides and phospholipids were separated from an hexane extract and their fatty acid composition determined by GLC as described above. Serum phospholipids were determined by enzymatic assay (Wako, Biolyon, France), triglycerides by UV test (Boehringer) and total cholesterol by CHOH–PAP. Fatty acid composition of serum total lipids was determined after saponification and hexane extract by GLC, and described above.

Statistical analysis

Data were analyzed by factor discriminatory analysis and Student's *t* test.

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* *Diet composition* (% dry matter): 48% corn starch, 24% sucrose, 18% casein supplemented with 0.4% DL methionine, 3% soybean oil, 2% cellulose, 4% salt mixture and 1% vitamin mixture in glucose.

Fatty acid composition (weight % of total methyl esters) determined by GLC as described in *Materials and methods section:*

C 14:0	0.3	C 18:2(n-6)	53.0
C 16:0	10.3	C 18:3(n-3)	6.8
C 18:0	4.2	C 20:0	0.3
C 18:1(n-9)	22.2	C 20:1(n-9)	0.2
C 18:1(n-7)	1.6	C 22:0	0.4
		C 24:0	0.2.

Results

The food intake was significantly decreased in the group receiving TCB, a compound known to lead to anorexia (12.9 ± 3 g/day · animal vs. 23.1 ± 0.09 g/day · animal; $P < 0.05$). However, the final body weights of the animals were not modified by the chemical treatments. Decreased feed intake did not result in lowered body weights due to the short time of exposure to the compound (4 days).

Table 1 shows the effects of the two PCBs on the lipid composition of the whole liver, the hepatic microsomal fraction and the serum. In the whole liver, TCB increased very significantly the amount of total lipids, which is due to an enhancement of the triglyceride content and can partly explain the enlargement of liver observed in this group. The ratio of liver weight to body weight is significantly increased by TCB (4.91 ± 0.29 % vs. 3.21 ± 0.38 %; $P < 0.01$). These modifications are in agreement with the TCB-induced fatty liver previously reported by other authors and did not occur in HCB-treated rats.

Hepatic microsomes were affected by both the treatments which caused an increase in cholesterol and phospholipid. The cholesterol/phospholipid ratio was slightly increased in TCB-treated rats relative to pair-fed controls whereas it is significantly decreased by HCB treatment. The pair feeding treatment also induced a large change in the cholesterol/phospholipid ratio. (*Table 1*).

No significant changes were seen in serum lipids in the treated groups, although a slight nonsignificant elevation of cholesterol was noticed (*Table 1*).

In *Tables 2* to *4* are presented the fatty acid composition of lipids of the liver, serum, and hepatic microsomes of TCB- and HCB-treated animals. Fatty acid composition of liver lipids revealed differences between the effects of the PCBs. HCB had no effect on total liver lipid composition, although TCB increased C 18:1 (n-9) proportion, which was balanced by a decrease of C 20:4(n-6). This is due to the modification of the triglycerides/phospholipids ratio reported above (see *Table 1*). Triglyceride composition was also differently affected by TCB, which decreased C 16:1 (n-7) and C 20:4 (n-6), whereas HCB had no clear effect. Phospholipid composition modifications involved the same kind of fatty acids. C 16:0 and C 18:0 were the most affected by the two compounds. TCB,

Table 1 Effect of TCB or HCB on lipid composition of rat liver, hepatic microsomes, and serum

	Liver (mg/g fresh tissue)			Hepatic microsomes			Serum (g/l)		
	Total lipids	Triglycerides	Phospholipids	Cholesterol phospholipids ug/mg protein		Cholesterol / phospholipids	Triglycerides	Phospholipids	Total Cholesterol
Control pair-fed	68.9	31.9	37.0	23.4	217.8	0.107	1.02	1.25	0.70
TCB-treated	109.2*	72.0*	37.2	36.3*	293.4*	0.123	0.99	1.44	0.87
Control *ad libitum*	67.7	32.3	35.4	28.2	187.2	0.154	1.05	1.36	0.66
HCB-treated	65.8	30.2	35.6	36.8*	297.0*	0.122*	0.84	1.36	0.82

* Denotes a significant difference from the corresponding control group ($P < 0.05$). Standard deviations do not exceed 12% of means.

Table 2 Effect of TCB and HCB on fatty acid composition of liver lipds. Results are expressed in weight % of total methyl esters. Control rats for TCB-treated are pair-fed.

	HCB-treated rats						TCB-treated rats					
	Total lipids		Triglycerides		Phospholipids		Total lipids		Triglycerides		Phospholipids	
	HCB	Control	HCB	Control	HCB	Control	TCB	Control	TCB	Control	TCB	Control
C 16 : 0	23.1	24.1	28.6	29.9	17.5*	18.4	22.6	24.7	25.7	28.5	14.1	18.3
C 16 : 1(n-7)	4.2	5.6	6.9	7.9	1.8	2.1	3.5	4.7	4.7*	7.6	1.0*	1.8
C 18 : 0	14.5	11.9	3.6	3.6	23.4*	20.0	11.2	14.3	4.0	3.6	28.1*	22.0
C 18 : 1(n-9)	18.1	16.9	33.4	31.1	4.7	4.6	28.9*	19.7	37.6	35.3	5.4	5.2
C 18 : 1(n-7)	4.6	3.7	5.4	4.5	3.6	3.4	4.1	4.4	4.6	4.9	2.7	4.0
C 18 : 2(n-6)	13.1	13.9	16.7	16.6	10.5	11.1	17.0	13.4	20.0	14.4	8.9	11.6
C 18 : 3(n-3)	0.9	0.8	1.5	1.4	0.1	0.1	1.4*	0.4	1.9	0.8	0.1	0.1
C 20 : 4(n-6)	16.6	17.5	3.0	3.8	29.5	30.5	8.9*	15.2	1.2*	3.8	31.0	29.1
C 22 : 6(n-3)	4.9	5.5	0.8	1.1	9	9.7	2.0	3.1	0.2*	1.0	8.8	7.8

* : $P < 0.05$; $n = 8$.
Standard deviations do not exceed 15% of means.

Table 3 Effect of TCB and HCB on fatty acid composition of liver hepatic microsomes. Results are expressed in weight % of total methyl esters. Control rats for TCB-treated rats are pair-fed

	HCB-treated rats						TCB-treated rats					
	Total lipids		Triglycerides		Phospholipids		Total lipids		Triglycerides		Phospholipids	
	HCB	Control	HCB	Control	HCB	Control	TCB	Control	TCB	Control	TCB	Control
C 16 : 0	20.5	21.9	34.1	37.2	18.9	20.8	17.1*	21.5	29.6*	32.2	16.3	19.8
C 16 : 1(n-7)	2.0*	2.8	2.4*	3.6	2.0	2.8	1.2	2.4	2.7	2.6	1.2	2.4
C 18 : 0	22.5*	19.2	31.4*	25.5	22.9*	20.7	26.0*	19.8	33.8*	29.2	27.2	19.6
C 18 : 1(n-9)	9.6	10.6	8.2	9.3	9.0	10.1	10.4	11.0	9.4	9.1	10.1	10.7
C 18 : 1(n-7)	3.7	3.5	3.4	3.5	3.4	3.5	3.0	3.9	2.7	3.6	2.8	3.7
C 18 : 2(n-6)	9.8	11.6	7.4	8.2	9.3	9.8	11.0	11.1	7.9	8.2	10.6	10.7
C 18 : 3(n-3)	0.2	0.3	0.2	0.3	0.2	0.3	0.5	0.2	0.4	0.2	0.6	0.2
C 20 : 4(n-6)	24.8	23.3	9.9	9.7	26.7	24.1	24.4	23.8	10.7	11.6	24.8	26.0
C 22 : 6(n-3)	6.8	6.7	2.9	2.7	7.6	7.4	6.3	6.2	2.8	3.2	6.4	6.6

* : $P < 0.05$; $n = 8$.
Standard deviations do not exceed 15% of means.

Table 4 Effect of TCB and HCB on fatty acid composition of serum total lipids

	HCB	Control	TCB	Control
C 14 : 0	1.0	0.5	0.5	0.5
C 16 : 0	21.2	21.5	19.1	21.4
C 16 : 1(n-7)	4.4	4.8	3.3	5.0
C 18 : 0	10.1	10.1	11.9	9.2
C 18 : 1(n-9)	15.1	17.0	18.3	18.5
C 18 : 1(n-7)	3.5	3.7	3.5	3.6
C 18 : 2(n-6)	15.1	15.4	14.0	15.5
C 20 : 4(n-6)	26.4	23.8	26.3	23.5
C 22 : 6(n-3)	3.0	3.1	3.1	2.7

Results are expressed in weight % of total methyl esters. Control rats for TCB-treated rats are pair-fed. Standard deviations do not exceed 15% of means.

but not HCB, was responsible for a clear increase of C 18:2 (n-6) in the triglycerides. In hepatic microsomes the significant modifications mainly concerned C 16:0. This fatty acid was decreased by the two compounds, which was compensated for by an increase of C 18:0. The same change could also be seen for serum lipids of TCB-treated animals.

Discussion

This study was designed in order to compare some aspects of lipid metabolism in rats treated with two prototypic PCBs. Our data confirmed the results of previous studies on the effect of complex mixtures of PCBs,[2-7] which showed alterations in lipid composition (i.e., liver lipid accumulation, changes in the cholesterol/phospholipid ratio, modifications of fatty acid composition). Because of the anorexia induced by TCB, each foreign compound was compared to its own control, which have received the same amount of food. Comparisons between the two compounds must take into account that the animals differ not only in the xenobiotic they received, but also in the amount of food they have eaten.

Some effects of TCB and HCB were similar. In microsomal lipids, both xenobiotics produced an increase of C 18:0, parallel to a decrease in C 16:0 proportion. This may suggest a greater activity of the elongation system of palmitoyl-Co A. The liver triglycerides accumulation occurred only in TCB-treated animals, and no concomitant increase in serum triglycerides was seen. This suggests an impaired exportation of the lipid material from the liver into the serum, which could be due to an altered delivery of very low density lipoproteins, as observed by other investigators in rats treated with Aroclor 1254.[22] Another specific effect of TCB was an increase of the proportion of C 18:2 (n-6) in triglycerides liver lipids. These observations might be explained by an alteration of the transport system of dietary lipids, which cannot be observed in our study because the rats were fasted before killing and the serum is lipoprotein-free. It is also possible that a decrease in enzymatic conversion of C 18:2 (n-6) to C 20:4 (n-2) occurs, since this latter fatty acid is decreased at the same time as the first one increased.

The cholesterol/phospholipid ratio was also affected differently by the two compounds. This ratio gives information about membrane fluidity, a concept which refers to the physical state of the lipid matrix and is related to the mobility of the proteins inside the membrane. It depends, among other parameters, on the level of unsaturation of the fatty acyl chains[23] and on the cholesterol/phospholipid ratio.[24] In the present study, food restriction in itself seemed to decrease this ratio. No significant changes were observed on the level of unsaturation of the microsomal fatty acids, since the variations occurred within the same class of fatty acids, but HCB led to a clear decrease of the cholesterol/phospholipid ratio. A similar observation was reported by Bernet et al.[25] for rats exposed to a mixture of polybrominated biphenyls, containing a high proportion of 2,2',4,4',5,5'-hexabromobiphenyl. According to Hinton et al.,[3] this decreased cholesterol/phospholipid ratio could be partly explained by retention of and an enhanced synthesis of phos-

pholipids. In contrast, TCB produced an increase in this ratio. Both the compounds simultaneously increased cholesterol and phospholipids, but not in the same porportions and the ratio was not affected in the same way.

These data suggest that the effects of PCBs on hepatic lipids are dependent on the structure of the PCB and subsequently may involve their mode of hepatic enzyme induction. Although the mechanisms are still unclear, special interest should be given to the effects of PCBs on lipid metabolism and transport, particularly the hepatic export of lipoproteins.

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